COMBINED TRANSMISSION AND SCANNING ELECTRON MICROSCOPY OF *IN SITU* PALAEOZOIC SPORES

by T. N. TAYLOR

ABSTRACT. This paper discusses pollen and spores isolated from Carboniferous reproductive organs, including fructifications belonging to lycopods, cordaites, and seed ferns. Microspores of the monosaccate genus *Endosporites* were macerated from sporangia of a single cone. Morphological variability ranges from grains still within the tetrahedral arrangement to solitary spores. Information is provided concerning saccus-corpus organization and exine ultrastructure, ornamentation, and stages of saccus ontogeny. Pollen grains included within the genus *Florinites* were examined in cordaitalean pollen sacs from different localities and stratigraphic levels. Both proximal and distal attachments between saccus and corpus are demonstrated. Spores of a new Pennsylvanian reproductive structure are described as consisting of a complex tectate exine supporting a verrucate ornamentation. Prepollen grains of the *Schopfipollinites*-type were isolated from a number of medullosan pteridosperm reproductive structures including the genera *Dolerotheca*, *Rhetinotheca*, *Aulacotheca*, *Whittleseya*, and *Halletheca*. Comparative studies of the spore exines suggest the occurrence of fundamental ultrastructural differences among the grains. Information is presented concerning the possible site of gamete emission from *Schopfipollinites* prepollen grains.

THE successful application of transmission electron microscopy to the study of fossil pollen exines by Ehrlich and Hall (1959) initiated a new era of palaeobotanical inquiry. With the subsequent availability of the scanning electron microscope and its ease of application in palaeontological research problems, researchers today are examining evidences of biological activity extending from the Precambrian to Recent, and including all levels of biological organization.

While dispersed spores and pollen grains found in rocks of Palaeozoic age have received a great deal of attention both for palaeontological and geological purposes, spores and pollen found in fructifications of known biological affinities have received little attention. It is the intent of the present paper to discuss the application of combined transmission and scanning electron microscopy to the study of *in situ* pollen, prepollen, and spores of Carboniferous (Pennsylvanian) age, and to demonstrate some of the types of information which have been made available utilizing these methods.

Studies of *in situ* pollen grains and spores are of particular importance because in most instances grains are present in sufficient numbers that ontogenetic differences may be separated from those which are truly taxonomic. This developmental approach may be undertaken with certain types of reproductive structures which mature in a sequential manner. The opportunity of sampling almost pure populations of spores, differing developmentally, from varying positions within a single cone provides an ideal means of studying spore wall ontogeny.

Because of the nature of this symposium the number of light photomicrographs used has been greatly reduced. It must be pointed out, however, that the use of light microscopy constitutes a valuable and indispensable aspect of any study in which the maximum number of pollen grain or spore features are to be elucidated.

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Technique. Pollen grains and spores were macerated from sporangia using dilute hydrochloric acid (2%), soaked in 12% hydrofluoric acid for 12 hours, and subsequently divided into three fractions. Grains to be examined by transmission electron microscopy were dehydrated to propylene oxide and embedded in Spurr low viscosity media. Sections cut at approximately 20 nm were poststained in a 5% aqueous solution of uranyl acetate for 20 minutes, followed by lead citrate. Grains to be examined with the scanning electron microscope were washed in two changes of distilled water, spread on standard SEM specimen stubs coated, while in suspension, with a thin film of dried silver conductive paint, and allowed to dry in air. The grains were then vapour coated with a thin film of gold and examined with a Cambridge Mark IIA instrument. Grains of the final sample were dehydrated to xylene and embedded in Harleco Synthetic Resin for examination by light microscopy.

In the case of very large grains, several hundred microns in diameter, such as specimens of *Schopfipollinites*, very satisfactory results have been obtained using the transmission-scanning operational mode (Swift and Brown, 1970). This technique enables entire sections to be examined in a single field of view. It consequently eliminates the necessity of constructing elaborate montages which are otherwise necessary for large grains because the minimum magnifications available with the transmission electron microscope are too high to record cell and wall component interrelationships. Sections ranging from 50 nm–1 μm were cut and mounted on single-hole slot grids covered with a thin collodion/carbon support film. Grids were subsequently stained with aqueous uranyl acetate followed by lead citrate and fitted into a special device mounted in the instrument stub holder.

The following is a discussion of four different spore types and the kind of information which has been assembled on their organization, morphology, and ultrastructure.

ENDOSPORITES

Endosporites Wilson and Coe 1940 is a monosaccate, trilete, microspore now known to be biologically related to some heterosporous members of the Lycophytina. The source of *Endosporites* specimens in previous studies has been from compressed, structureless cones, or from *sporae dispersae* assemblages. The material presented here comes from calcium carbonate petrifactions collected from probable lower Pennsylvanian sediments in eastern Kentucky (Good and Taylor 1970). Of particular

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EXPLANATION OF PLATE 96

Fig. 1. Transmission electron micrograph. Composite reconstruction of three spores of *Endosporites* tetrad. Arrows indicate positions of apical papillae. ×510.

Fig. 2. Partial lateral view of monosaccate spore showing thickened corpus wall (left) with external ornamentation, and internal saccus reticulations. ×2700.

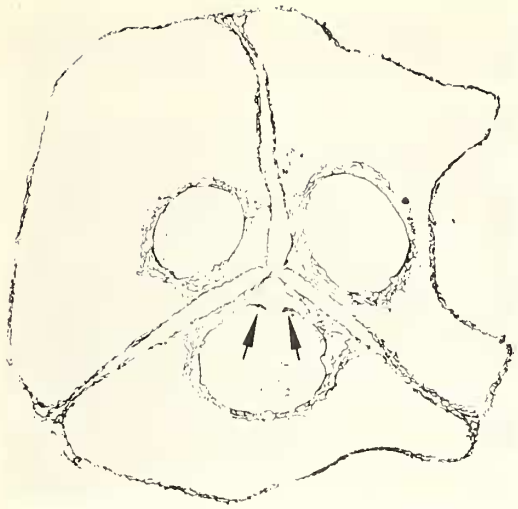
Fig. 3. Three spores of *Endosporites* tetrad showing relationship between corpus and saccus wall. ×2100.

Fig. 4. Scanning electron micrograph of *Endosporites* tetrad showing three spores, with the fourth represented by the corpus and a remnant of the saccus. ×500.

Fig. 5. Lateral view of *Florinites* grain showing proximal and distal saccus attachment, and internal saccus reticulations. ×1000.

Fig. 6. Limbus and proximal surface of *Endosporites* spore. ×4750.

Fig. 7. Immature *Endosporites* spore prior to saccus enlargement. ×3300.



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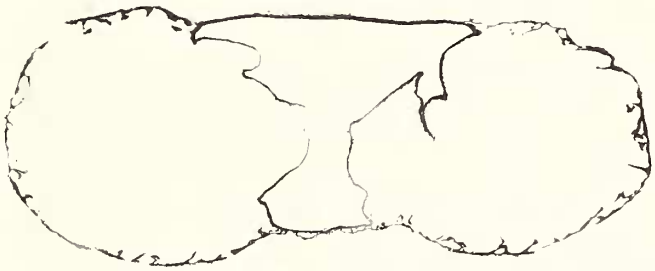

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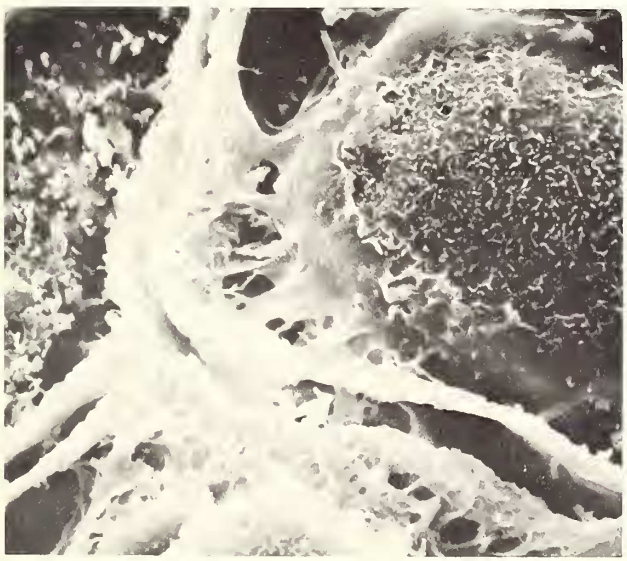

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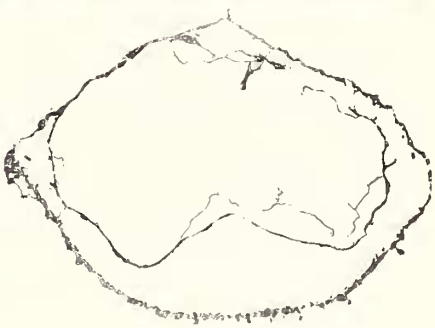

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importance with this material is the fact that within certain sporangia various stages of spore ontogeny are represented, including spores still within the tetrahedral arrangement (Pl. 96, fig. 1).

One of the most obvious advantages of transmission and scanning electron microscopy in the study of pollen grains and spores lies in the ability to discern more accurately and interpret correctly, complex ornamentation patterns which may be present on both the internal and external exine surfaces. Pl. 96, fig. 6 shows the distal surface of a spore ornamented by closely spaced, blunt-tipped spinules which are basally fused to form a reticulum. Along the equatorial rim of the spore, where the proximal and distal faces of the saccus become continuous, the spinous projections are fused, and together with the loosely arranged exinous strands, provide a thickness to the limbus. The proximal surface of *Endosporites* is uniformly smooth, interrupted only by irregularly shaped and randomly disposed pits (Pl. 96, fig. 6). Such features are too small to be resolvable with light microscopy, and are extremely difficult to characterize by transmission electron microscopy.

Pl. 96, fig. 4 shows three complete spores of a tetrad and the fourth represented by the corpus and a small remnant of the saccus wall. Spores with the saccus fragmented and torn allow observations to be made of the internal saccus surface and show irregular reticulations of the wall, as well as ornamentation of the corpus.

One of the conspicuous features of *Endosporites* microspores from the Kentucky locality when examined by light microscopy is the occurrence of three apical papillae (= interradial papillae) that appear as small dark crescent-shaped objects close to the spore centre; one between each of the laesurae. Transmission and scanning electron microscopy define the nature and relationship of these structures to the saccus and corpus walls. The lowest spore in Pl. 96, fig. 1 shows two of the apical papillae separated by a shallow depression and occurring on the inner surface of the corpus wall; but not arising from the outer saccus wall as has been previously thought.

Ultrastructure and exine stratification of *Endosporites* are best illustrated in Pl. 96, fig. 1 where the relationship between the wall of the corpus and saccus is apparent. The exine is constructed of two layers, with units comparable to the topographic equivalents 'nexine' and 'sexine'. In *Endosporites*, corpus-saccus attachment occurs only at the proximal pole (Pl. 96, figs. 1, 7). In the region where the corpus and saccus are fused on the proximal surface the wall is quite thick (3 μm) and consists of a series of anastomosing exinous strands. Pl. 96, figs. 1, 3 illustrates the proximal continuity provided between saccus and corpus by these delicate strands as viewed in both the transmission and scanning modes.

The spores macerated from the sporangia range from 73 to 121 μm in diameter; however, some of the microspores are appreciably smaller (21 μm). A large number of these smaller spores lack trilete scars and it has been suggested that these may represent isolated central bodies. Other small spores of a similar diameter, but possessing a well-defined trilete mark and in some instances apical papillae, are further distinguished from the more typical *Endosporites* grains by a more highly ornamented distal surface and the absence of a clearly defined saccus (Pl. 96, fig. 7). Transmission micrographs of this latter spore type show apical papillae and a reduced saccus surrounding the central body. These two features suggest a level of spore ontogeny in which the saccus is just beginning to differentiate through the

separation of the two wall layers. A fully developed saccus in *Endosporites* appears like the configuration of the spores in Pl. 96, fig. 1.

FLORINITES AND *FLORINITES*-TYPE POLLEN

The genus *Florinites* Schopf, Wilson, and Bentall 1944 was instituted for dispersed monosaccate pollen grains of presumed cordaitalean affinity. The generic diagnosis has been difficult to apply, principally because of the confusion regarding grain morphology and structural organization. With the improved techniques now available, new information has been obtained on the morphology of this type of grain. Specimens used were macerated from *Cordaianthus* pollen sacs found in coal balls collected near West Mineral, Kansas (middle Pennsylvanian), and in eastern Kentucky.

Florinites grains are monosaccate, and consist of a spherical central body (= corpus) surrounded laterally by a large, internally reticulate, air bladder (= saccus). In polar view the saccus appears circular–elliptical, with the corpus typically circular in outline. In several morphological studies of cordaite pollen the grains are described as consisting of a large air sac which completely encircles the internal corpus. In these studies body-bladder attachment has been described as occurring only at the distal pole. Combined transmission and scanning electron microscopy demonstrate that the attachment between the body and the bladder occurs at both the proximal and distal poles (Pl. 96, fig. 5). On the proximal surface body-bladder attachment is approximately equal to the maximum body diameter, whereas distally this attachment is typically less than body diameter. Distally the body-bladder attachment may assume a variety of configurations, varying from small and irregular to large and highly angular. In some instances attachment may approach a configuration which superficially resembles a sulcus. On the proximal surface of the grain attachment appears to be more regular and conforms to the symmetry of the central body. Thus in the case of the monosaccate grain *Florinites* the structure consists of a central body totally enclosed by a bladder and fused to it on both the proximal and distal surface.

External bladder ornamentation is best described as laevigate when viewed with the light microscope. When examined with the SEM, the bladder appears as a series of irregular depressions corresponding to the outlines of the internal bladder reticulations (Pl. 96, fig. 5). The ability to examine a fractured internal or enclosed grain component, in this instance the external surface of the corpus, provides information which would be impossible to discern with light microscopy (Pl. 97, fig. 5).

Florinites grains demonstrate the same apparent bladder ontogeny characteristic of extant saccate pollen in which the bladder arises by a separation between the sexine and nexine. Small ridges which characterize the external surface of the central body on the lateral walls represent former regions of exine attachment prior to saccus inflation (Pl. 96, fig. 5). The nexine, which is typically lamellated in extant saccate pollen, shows no observable ultrastructural layering in the *Florinites* specimens.

Another monosaccate pollen grain resembling *Florinites* in many morphological features was also recovered from *Cordaianthus* pollen sacs collected at the eastern Kentucky locality. The grains are larger than the *Florinites* specimens, ranging in size from 115 to 180 μm. Both radially symmetrical and bilaterally symmetrical specimens were found. Grains which show a radial organization are exclusively

trilete, while the bilateral specimens have a suture organization of the monolete type. Most grains show varying degrees of suture expression between these two types. In this grain saccus-corpus attachment also occurs at both the proximal and distal poles.

Externally the saccus is psilate except in the region of the proximal pole. Pl. 97, fig. 6 indicates that the region of the trilete mark is complex, consisting of a series of uneven muri which are most prominent closest to the suture, decreasing in size away from the laesurae. The trilete mark consists of a narrow Y-shaped depression supporting a median, elevated ridge. The central ridge appears to arise from the base of the depression, and is supported by delicate ribs which are uniformly situated at right angles to the long axis of the laesura.

Internally, the saccus of this grain is ornamented by a network of inwardly projecting wall thickenings which form a reticulate pattern, and appear similar to the ornamentation of *Florinites*. The external corpus wall is ornamented by a delicate reticulum (Pl. 96, fig. 2). In section view the projections of the reticulum appear as finely spaced, uniformly developed processes which at some levels appear to bifurcate at their tips (Pl. 96, fig. 2). These processes appear continuous with the saccus reticulum and constitute an ontogenetic feature resulting from the separation of the sexine early in the formation of the saccus. The corpus wall is quite thick, measuring approximately 2 μm (Pl. 96, fig. 2). Ultrastructurally, this layer is composed of a series of irregularly thickened lamellations which may be up to 0·2 μm thick (Pl. 97, fig. 4).

TECTATE GRAIN

The grain illustrated in Pl. 97, fig. 1 was extracted from a reproductive organ consisting of numerous thick-walled sporangia which are attached to vascularized bract-like structures by elongate pedicels (Taylor 1972). The spores are trilete and circular—subcircular in outline. They range from 38 to 55 μm in diameter and are characterized by trilete rays which extend approximately three-quarters of the spore radius. The spore illustrated in Pl. 97, fig. 1 shows the elevated and prominent nature of the trilete mark. Ornamentation consists of a series of irregular verrucae which extend up to 1·4 μm high (Pl. 97, figs. 1, 2). When examined by light microscopy the surface of the verrucae appear to bear slight depressions. Ultrathin sections of the spores indicate, however, that this pattern is the result of

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EXPLANATION OF PLATE 97

Fig. 1. Proximal surface of Pennsylvanian spore showing verrucate ornamentation and trilete mark. ×1200.

Fig. 2. Fractured surface of spore in Fig. 1 showing level of exine organization and relationship of wall layer components. ×6000.

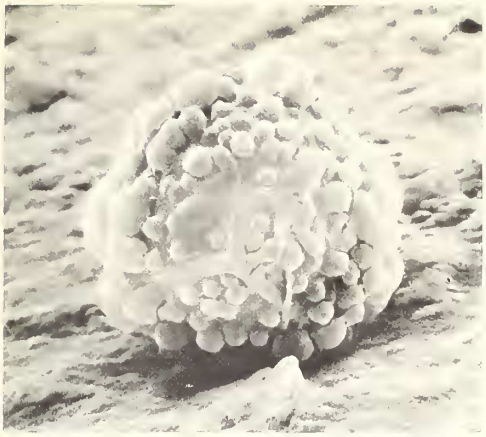
Fig. 3. Transmission electron micrograph of Fig. 1 spore exine. ×8500.

Fig. 4. Transmission electron micrograph of corpus wall of monosaccate spore in Pl. 96, fig. 2, showing nexine lamellations. ×15000.

Fig. 5. Scanning electron micrograph of *Florinites* grain showing ruptured saccus and ornamentation of corpus. ×5900.

Fig. 6. Proximal region of monosaccate grain showing complex organization of trilete mark. ×900.

Fig. 7. Transmission electron micrograph of spore in Fig. 1 showing wall stratification. Interruptions in the nexine indicate the position of two laesurae of the trilete mark. ×1400.



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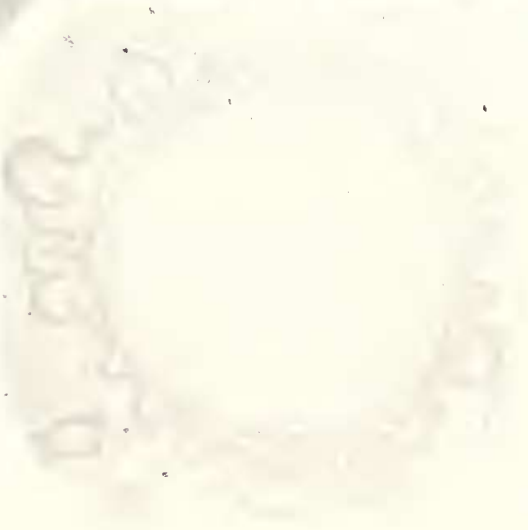

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exine organization rather than surface features of the verrucae (Pl. 97, figs. 3, 7). The section of the spore illustrated in Pl. 97, fig. 7 is slightly oblique in the proximal-distal plane so that two of the laesurae appear as interruptions in the inner component of the wall.

The exine of the grain consists of four easily delimited layers. Using the terms commonly applied in pollen exine organization, these may be called tectum, columellae, pedium; and some level of nexine development (Pl. 97, figs. 3, 7). The columellae extend from the foot layer or pedium, but are not in contact with the tectum at their distal ends. The tectum appears to be attached along the sides of the columellae, rather than at the distal end of each structure (P. 97, fig. 7). The broken surface of the spore wall illustrated in Pl. 97, fig. 2 clearly shows the relationships of the exine components, and further clarifies that the tectum is not fused with the pedium between the columellae. The nexine, or inner preserved layer of the spore wall, is uniform in thickness except in the region of the trilete mark where some thickening is present (Pl. 97, fig. 7).

SCHOPFIPOLLINITES

The genus *Schopfipollinites* Potonié and Kremp 1954 (= *Monoletes*) has been used for large (100–500 μm) bilaterally symmetrical pollen grains of the prepollen type, thought to be produced by medullosan pteridosperms. The grains are typically characterized by a single proximal suture having a slight angular deflection (Pl. 98, fig. 2). On the distal surface two longitudinal grooves separated by a median ridge (umbo) are occasionally present; however, the occurrence of this ridge does not appear to be a constant feature of the taxon. Exine ornamentation as examined by transmitted light is typically described as minutely granulose-reticulate, or smooth. Pl. 98, fig. 6 is a light photomicrograph of what appears to be the external ornamentation pattern of a grain extracted from the microsporangiate organ *Halletheca* (Taylor 1971). The scanning electron micrograph of a *Halletheca* grain (Pl. 98, fig. 3) shows that the surface is highly variable in ornament, and that the apparent reticulate appearance of the wall in Pl. 98, fig. 6 is a feature of the internal organization of the wall rather than of surface topography. In some grains, especially in the region of the suture, exine deposition was not complete at the time of fossilization

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EXPLANATION OF PLATE 98

Figs. 1–8. *Schopfipollinites* grains.

Fig. 1. Distal view of grain macerated from *Dolerotheca* fructification. ×204.

Fig. 2. Proximal view showing suture with median deflection. ×216.

Fig. 3. Portion of *Halletheca* grain showing internal lumina. Compare with organization illustrated in Fig. 6 obtained with light microscopy. ×4950.

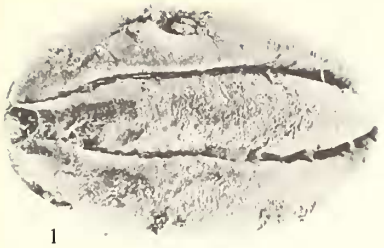
Fig. 4. Internal organization of exine of grain macerated from *Schopfitheca* reproductive organ. ×5600.

Fig. 5. Transmission scanning micrograph of grain showing region of proximal suture and distal umbo. Note thickened wall in proximal suture region. ×4000.

Fig. 6. Light photomicrograph of grain macerated from *Halletheca* sporangium. ×2500.

Fig. 7. Proximal surface of grain showing incomplete deposition of exine. ×965.

Fig. 8. Electron micrograph of grain macerated from *Schopfitheca* reproductive structure showing internal exine organization. Compare with organization of grain in Fig. 5. ×5500.



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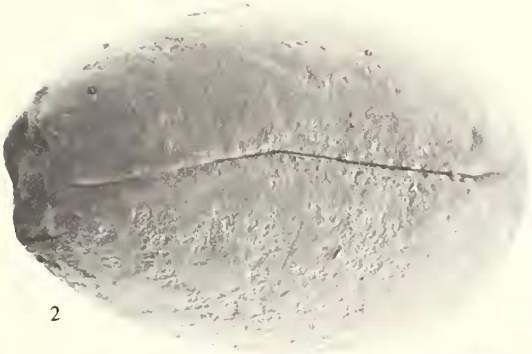

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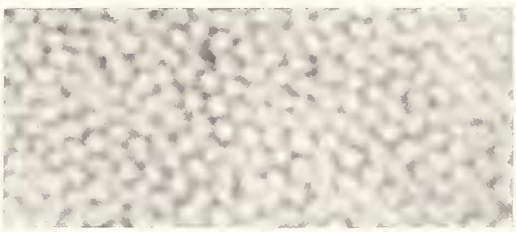

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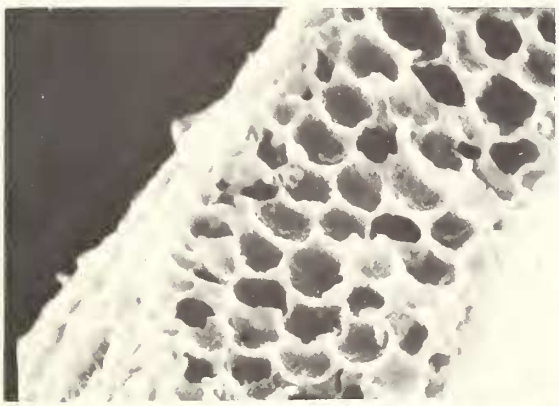

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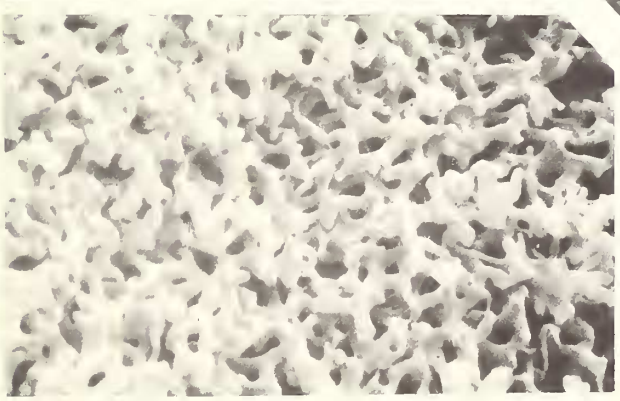

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so that internal exine organization is easily visible without fracturing or sectioning the spore wall (Pl. 98, fig. 7). Grains of this type further point out the necessity of a combined approach to the elucidation of spore wall features.

To determine the constancy of this form of exine organization and its potential use as a taxonomic feature in dispersed spores, specimens of *Schopfipollinites* were extracted from a number of medullosan pollen organs (*Dolerotheca*, *Rhetinotheca*, *Aulacotheca*, *Whittleseya*) and compared with the organization found in *Halletheca*. While a number of *Schopfipollinites*-containing reproductive organs have been described, only very few are known from structurally preserved specimens. Consequently, the opportunity accurately to delimit reproductive organs differing in preservational mode by the identification of their spores is of considerable importance to palaeobotanical systematics.

One such structureless reproductive organ containing *Schopfipollinites* grains is the genus *Schopfitheca* (Delevoryas 1964). The specimen consists of a stalked, clavate-pyriform microsporangiate structure approximately 20 mm long. Ultrastructurally, the wall of the prepollen grain consists of a thickened foot layer from which arise a series of anastomosing baculae (Pl. 98, fig. 8). At some levels these units are fused and demonstrate an organization similar to that in *Halletheca* or *Dolerotheca* grains (Pl. 98, fig. 5). The scanning electron micrograph of the fractured surface of one of these *Schopfitheca* grains (Pl. 98, fig. 4) indicates clearly a distinct difference in internal exine organization from the *Halletheca* grain.

One approach to the problem of identifying stages in exine development is the use of stereomicrography and photogrammetric analysis (Boyde 1970), which enables one to view and accurately measure structural components of the wall. The capability of making stereo pictures with the scanning electron microscope not only provides for an accurate three dimensional representation of the exine organization, but provides a means of making precise parallax measurements of these three dimensional structures. It has already been possible to correlate changes in exine thickness with distinct differences in structural configuration, and to correlate these in turn with grain maturity as determined by such additional features as level of sporangium ontogeny. Preliminary information on the internal wall organization of *Schopfipolliuites* prepollen grains extracted from different reproductive organs, as well as on those of differing preservational modes, appears to show that structural differences in exine organization exist. Whether these differences represent stages in wall ontogeny or are solely taxonomic must await continued investigation.

The internal organization of fossil pollen and spores may also provide important information on other aspects of the plants that produced them; for instance, the mode of gamete emission in prepollen grains of the *Schopfipollinites*-type. Germinal exit in such grains has generally been regarded as occurring from the proximal suture (see Chaloner 1970 for an excellent review of this problem), although Renault (1896) suggested evidence of distal germination in grains produced by *Dolerotheca fertilis*. An examination of the proximal suture of a *Dolerotheca* grain (Pl. 98, fig. 5) indicates that although the total thickness of the grain wall is reduced by approximately one-third, the floor and wall adjacent to the suture are distinctly thickened. The suture would therefore seem an unlikely site for gamete emission. On the distal surface, however, the exine is appreciably thinner beneath each of the distal grooves, and super-

ficially appears a more probable exit site. Relative exine thickness, when compared in a large number of Palaeozoic prepollen and pollen types, may provide cumulative information on germination mode and evolutionary trends associated with the process of fertilization.

CONCLUSION

The examples included in this paper illustrate the value of combined transmission and scanning electron microscopy to the study of pollen grains and spores present in reproductive organs. The SEM thoroughly delineates features of the internal and external exine surfaces, and also provides a means whereby developmental features may be critically studied. Transmission electron microscopy provides information about the ultrastructural organization of the wall, as well as supplementing information obtained by the SEM. There is little doubt that basic structural and functional differences are present in the walls of spores and pollen grains produced by various types of Palaeozoic vascular plants. Such differences may be correlated to provide information on the evolutionary relationships between seemingly diverse taxa, as well as providing a means of determining the biological origin of various dispersed spores and pollen grains.

Acknowledgements. The author is indebted to Sheila D. Brack, Department of Botany, Ohio University, and Michael A. Millay, Department of Biological Sciences, University of Illinois at Chicago Circle, and to the American Philosophical Society and National Science Foundation (GB-35958) for financial assistance.

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Discussion on Dr. Taylor's paper:

Kempf: Dr. Taylor has extremely fine material from the Palaeozoic. In some of the spores it was quite obvious that two layers have been preserved, a thin inner layer and a thicker outer layer, which occasionally showed zonations. I would regard these layers as exine and perine. The saccus was always derived from the outer layer—it was not between the two layers, but within the outer layer—the perine. Did you also section *Equisetum*? What is the fine structure of the wall?

Taylor: The wall of *Equisetum* is very nondescript with really no fine structure at all.

Kempf: You know the work of Gullvåg, who is studying *Equisetum*. She has shown a tubular fine structure (which may be artefactual). Perhaps there is some fine structure of this kind?

Taylor: I don't know. I would like to comment on this perine problem. I'm not too concerned with what we call the layers of the walls, because in the Palaeozoic we have very little to compare the structures with. If we could compare with living plants, and show that the perine was present on the living form, then that would be a different matter. The other point is, the perine is the layer that is put on last. It is a developmental term really, and again, working with Palaeozoic material, we are not really in a position to put names to the different layers. If one is to make such distinctions, they must be made at the biochemical level, but at our present state of knowledge we are in no position to do this kind of work.

Kempf: At one time you were describing a wall with four layers; but there should be only two layers, and three of these layers are zonations of the outer layer. Also the perine is not formed last, it is a primary layer, and it is formed first, then the exine and then the intine. You can observe this optically, and sometimes you find spores with only the perine and the exine present and no intine.

Taylor: But how can you demonstrate that what you are calling the intine is not just another layer of the exine without doing biochemical tests? It seems to me rather a matter of semantics.

Skelton: Have you carried your research back to Silurian plants—prespore plants—to see if you can get any structure from them?

Taylor: No, I have restricted my work to the Upper Palaeozoic.